HETEROCHROMOSOMES IN THE GUINEA-PIG.

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In view of the fact that the guinea-pig is so much used for experimental breeding tests in Mendelian heredity, it seemed desirable to secure some further knowledge of the behavior of the chromatin in the germ cells. An attempt has therefore been made to follow through the spermatogenesis, and a brief note on the subject was published in the BIOLOGICAL BULLETIN, Vol. XX., No. 1, January, 1911. The material has proved to be very unfavorable, and the results are not so satisfactory as are to be desired. As Meves ('99) has given a very full account of the transformation of the spermatids into spermatozoa, I shall omit all discussion of that phase of the spermatogenesis, and confine my attention to the behavior of the chromatin in the nuclei and mitoses of spermatogonia, spermatocytes and spermatids.

METHODS.

At first the testes were fixed by several methods which had given good preparations of other spermatogenesis material. It soon became evident that only the Flemming and Hermann osmic mixtures gave even fairly good fixation of the chromosomes in mitosis, and also that the testes must be removed from the animal, cut into small pieces, and transferred to the fixing fluid with great rapidity in order to secure good results. Meves' method of using the fixing solutions at 35° C. proved to be an improvement on the use of cold solutions. The sections, 5μ thick, were stained with either thionin or iron-hæmatoxylin, orange G. being used in some cases as a plasma stain. Thionin, or thionin and orange gave the most satisfactory results, especially for the chromosomes in mitosis. Aceto-carmine preparations were made for each set of fresh material and proved valuable. It was found that pieces of the testis macerated with needles in aceto-carmine would keep in fairly good condition in a tightly stoppered vial for several weeks.

SPERMATOGONIA.

As in other vertebrates the testes consist of much coiled tubules in which, in mature animals, spermatogonia and Sertoli-cells are found at the periphery against the wall of the tubule; then come first and second spermatocytes in various stages, spermatids, and unripe spermatozoa attached to long processes extending from the Sertoli-cells toward the center of the tubule, ripe spermatozoa being found in or near the lumen of the tubule.

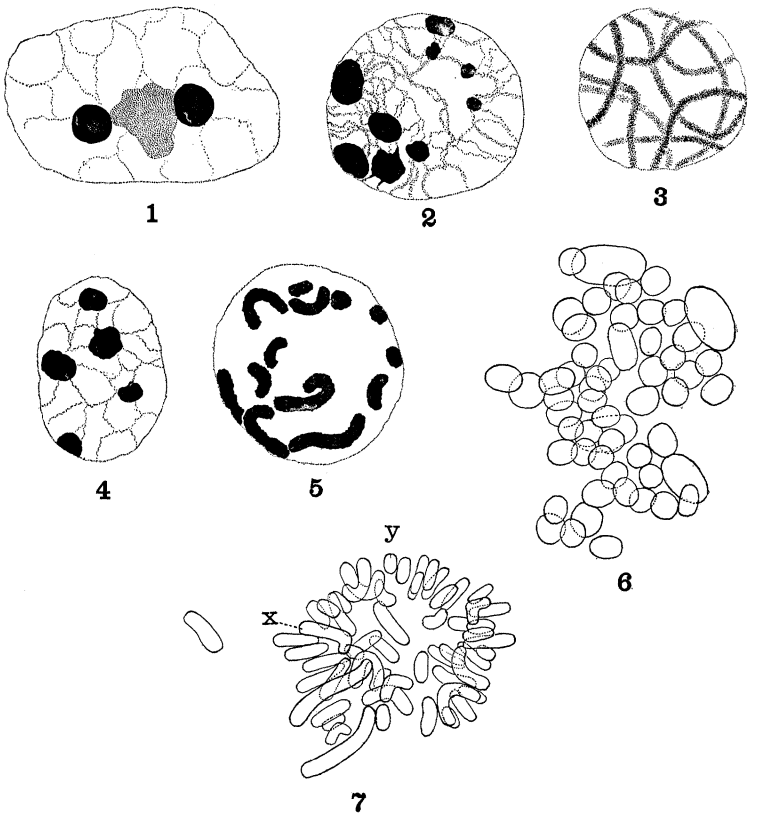


FIG. 1. Nucleus of Sertoli cell.

FIG. 2. Nucleus of spermatogonium.

FIG. 3. Spireme stage, spermatogonium.

FIGS. 4 and 5. Nuclei from a young testis, rest-stage and prophase.

FIG. 6. Fifty-six chromosomes of a spermatogonial prophase. Aceto-carm. prep.

FIG. 7. Equatorial plate of a spermatogonial mitosis; 56 chromosomes. All figures 2,000 × 1½, reduced ½.

In one very young animal only spermatogonia were found, Sertoli-cells not having been differentiated, and no maturation stages having yet appeared.

The nuclei of the Sertoli-cells (Fig. 1) are clearly differentiated from those of the spermatogonia. They are larger and usually flattened parallel with the wall of the tubule. In the center of the nucleus is a large plasmosome and closely associated with it one, two or more chromatin nucleoli, or karyosomes, which often appear to have a central vacuole. The reticulum of the nucleus does not stain with thionin or iron-hæmatoxylin.

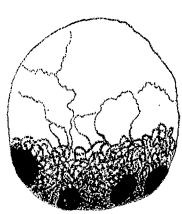
The larger number of the spermatogonial nuclei contain large clumps of chromatin of various sizes, and tangled threads into which the clumps are gradually resolved after a mitosis (Fig. 2). Shortly before a mitosis all of the clumps disappear and a perfect spireme stage is formed (Fig. 3). In a young testis nearly all of the nuclei are similar to Fig. 4. A few are in spireme or prophase stages (Fig. 5). It has proved a very difficult matter to determine the number of chromosomes either in the spermatogonia or spermatocytes. I have never found a case of a perfectly flat plate where there was no overlapping. After spending a great deal of time over this one point, I have concluded that 56 (Figs. 6 and 7) is probably the correct number. Fig. 6 shows in outline the chromosomes of a prophase, from an aceto-carmine preparation, the cell having been much flattened. Two large pairs can be distinguished, and the others vary considerably in form and size. Fig. 7 is the best equatorial plate which was seen either in aceto-carmine preparations or in sections. This was taken from Hermann material stained with thionin. It will at once be seen that the chromosomes overlap in such a manner as to make accurate counting difficult. The one aberrant chromosome is not a characteristic feature. One unusually long pair of rods is conspicuous. There are several V's, and the remainder have the form of straight or somewhat curved rods. The two long rods are always noticeable in an anaphase, lagging behind the shorter chromosomes. The V's are probably the same chromosomes which have the spindle-fibers attached centrally and pass to the poles as V's in the first maturation mitosis. The heterochromosomes one cannot distinguish at this stage with

certainty; the smaller one is one of the smallest chromosomes, perhaps *y*, and *x* which seems to have no mate may be the larger.

First Spermatocytes.

The nuclei of the youngest spermatocytes are similar to those of the spermatogonia (Fig. 2), clumps of chromatin and fine threads. Before the clumps have disappeared the chromatin threads are more or less massed at one side of the nucleus (Fig. 8) in a synizesis stage, which resolves itself into an irregular bouquet stage (Fig. 9) with loops much coarser than the threads of the previous stage. Frequently one dense chromatin mass is seen half hidden among the bases of the loops. Some of the loops look as though formed by telosynapsis, but if this is so, the halves of the bivalent loops must later become parallel, for the bouquet stage passes over into a parasynaptic stage (Fig. 10 and Fig. 11) which is followed by a spireme stage (Fig. 12) in which there is no longer any evidence of synapsis. In the spireme stage there are always small masses of dense chromatin-like material lying against the nuclear membrane, and staining as deeply with thionin as the chromosomes in mitosis. A large heterochromosome *x* is always present, and one or more plasmosomes can usually be found in sections. In early prophases (Figs. 13 and 14) the chromatin appears as though gathering together about definite centers along the spireme, leaving linin threads between. Sometimes a longitudinal split is visible in some of the larger chromsomes (Fig. 14.) In several cases at this stage the two heterochromosomes (*xy*) have been clearly separated as in Figs. 13 and 14. In Fig. 13 a pale plasmosome is also present.

In most first spermatocyte spindles two chromosomes are conspicuous (Fig. 15). One of these (*xy*) is the unequal heterochromosome, usually bilobed at the larger end (*x*). The other (*a*) is larger than any of the others and without doubt is composed of the two long rods of the spermatogonia. As a means of determining the reduced number of chromosomes, a number of spindles in the best material were selected, and an attempt was made to draw all of the chromosomes which were divided between two sections. Twenty-eight were found in a number of the clearest cases. These are all shown for the spindle of



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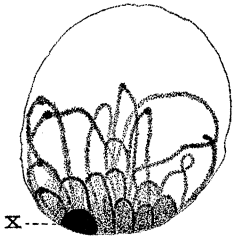

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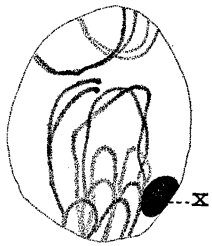

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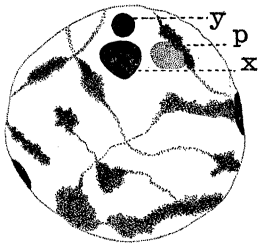

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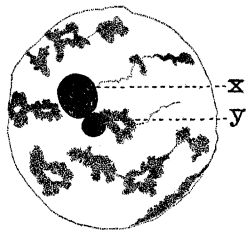

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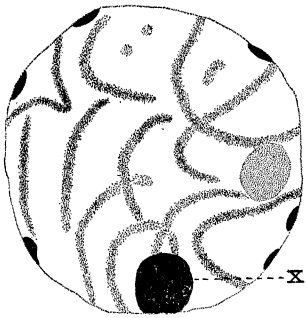

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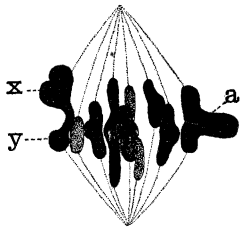

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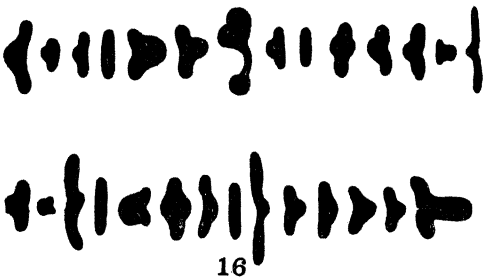

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FIG. 8. Synizesis stage.

FIG. 9. Bouquet stage; *x*, the heterochromosome.

FIGS. 10 and 11. Later stages showing parasynapsis.

FIG. 12. Spireme stage.

FIGS. 13–14. Prophases showing the unequal pair of heterochromosomes (*x*, *y*).

FIG. 15. First spermatocyte metaphase; *x*, *y*, the heterochromosome pair; *a*, the largest chromosome.

FIG. 16. The 28 chromosomes from the spindle of Fig. 15.

FIG. 17. First spermatocyte equatorial plate, 28 chromosomes.

which Fig. 15 is a part, in Fig. 16. Many of them are evidently parasynaptic pairs attached to spindle fibers at one end, others are similar pairs attached in the middle and pulling apart in the

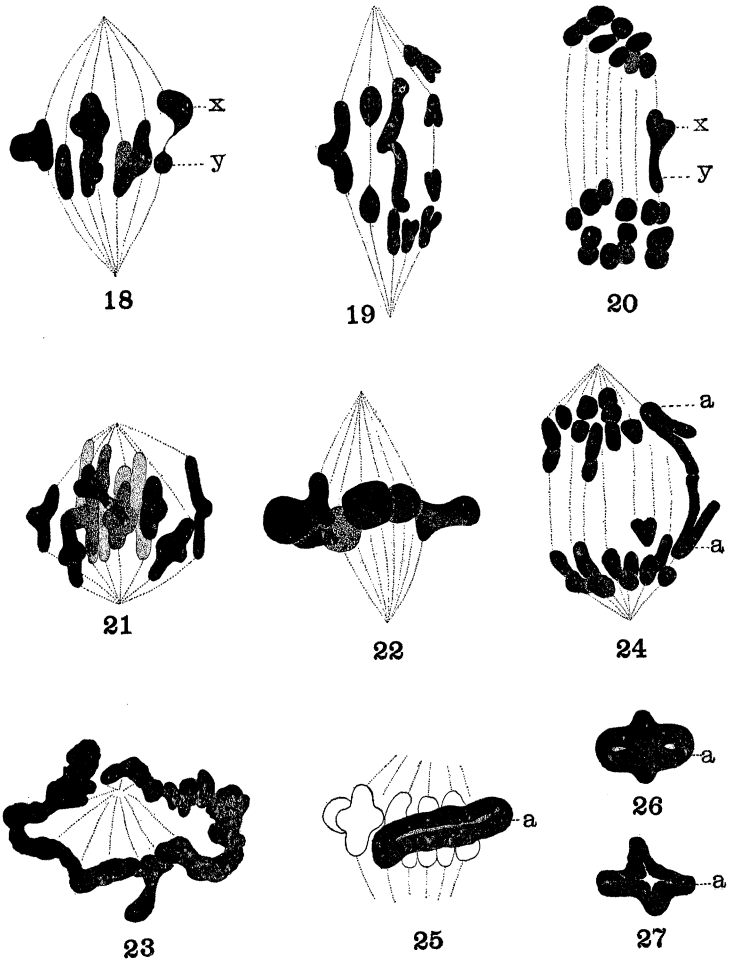

FIG. 18. The heterochromosome pair (*x y*) dividing.

FIG. 19. Metakinesis.

FIG. 20. Anaphase. Heterochromosome pair still undivided.

FIG. 21. Newly formed spindle; chromosomes well advanced in metakinesis.

FIG. 22. Older spindle; metakinesis hardly begun.

FIG. 23. Spindle forming with chromatin in form of a thick spireme.

FIG. 24. Anaphase; chromosome *a* separating as a pair of *V*'s.

FIGS. 25–27. Chromosome *a* in the form of a split band, passing into a cross-form in metakinesis.

form of crosses. The rods are probably lateral views of cross-shaped forms. Fig. 17 shows 28 chromosomes in an equatorial plate. Fig. 18 is another metaphase in which the members of the heterochromosome pair are already separating earlier than the others. In Fig. 19 the separation of some of the chromosomes as rods and V's in metakinesis may be seen. Fig. 20 is a late anaphase in which the heterochromosome pair is still undivided. This is unusual, but shows a kind of variation which is noticeable through all the stages of the spermatogenesis. Variation is most conspicuous in the prophases of the first maturation mitosis. As a rule one finds such early prophases as are shown in Figs. 13 and 14, and the chromosomes of the young spindle (Fig. 21) are well on their way in the process of metakinesis, but one sometimes finds the chromosomes of an older spindle (Fig. 22) much less far along toward division than in Fig. 21, and not infrequently I have seen a condensed spireme, as in Fig. 23, free in the cell, the nuclear membrane having disappeared, and a spindle forming. These variations must, I think, be an expression of the difference in rate at which maturation takes place at different times, and probably under different stimuli, or stimuli of different intensity. Fig. 24 is an interesting anaphase showing the large chromosome pair separating as a pair of V's instead of a pair of rods, as would have been expected from Fig. 15*a*. In the preparations from one animal I repeatedly saw the largest chromosome as shown in Figs. 25, 26 and 27, a split band forming a cross in a later stage (Fig. 27). In all other material the largest chromosome has appeared as in Fig. 15, which, together with Fig. 24, suggests that the four ends of the two long chromosomes which form the pair are usually folded together. The two lateral halves of the cross shown in Fig. 27 folded together would give the form shown in Fig. 15*a*, and might then separate as in Fig. 24. The same kind of folding has very likely occurred in chromosomes 5 and 19 in the series shown in Fig. 16, and possibly in other cases where it is less evident.

Second Spermatocytes.

Between the two maturation mitoses there is a rest stage in which the pairs of second spermatocytes may often be found

connected by spindle fibers as in Fig. 28. This figure shows the larger (x) and smaller (y) heterochromosome with smooth outline, and the remainder of the chromatin in the form of irregular clumps and strands. In some cases second spermatocyte spindles have been found associated with first spermatocytes

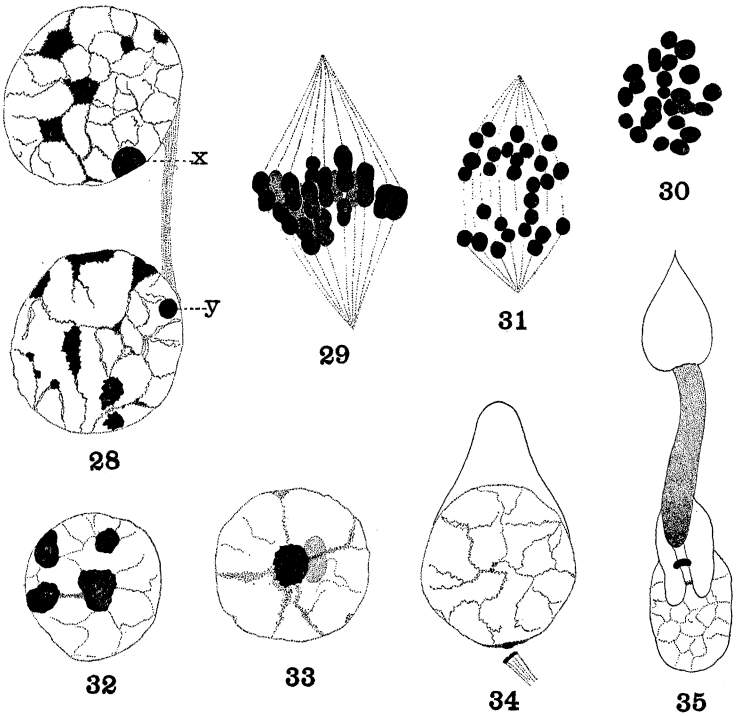

FIG. 28. A pair of second spermatocytes showing the heterochromosomes x and y.

FIG. 29. Second spermatocyte metaphase.

FIG. 30. Second spermatocyte equatorial plate.

FIG. 31. Second spermatocyte anaphase.

FIGS. 32–35. Four stages in the transformation of the spermatid, showing no trace of the heterochromosomes. All figures 2,000 × 1½, reduced ½.

in mitosis in such a way as to indicate that the rest stage between the two divisions may often be omitted. This is as far as the heterochromosomes can be traced.

In the second maturation spindle I have never been able to count the full number of chromosomes because of crowding and overlapping. In the clearest cases seen in aceto-carmine prepa-

rations, the chromosomes are dumb-bell shaped, as in Fig. 29. They are, however, rarely so well separated. Fig. 30 shows 24 out of the 28 which should appear in such a plate, and Fig. 31 an anaphase in which no attempt was made to draw all of the chromosomes. These figures, though valueless as a demonstration of the number of chromosomes or of the division and distribution of the heterochromosomes, are given to show that in the guinea-pig there is no such second synapsis or numerical reduction as has been described by Guyer ('09, '10) for the guinea-chicken, the domestic chicken and for man, also by Jordan ('11) for the opossum. There are plenty of figures in the sections which show that the number is certainly not reduced one half, and probably not reduced at all.

The Spermatids.

In the youngest spermatids as in other stages immediately following mitosis, the chromatin appears in irregular clumps (Fig. 32). The most characteristic spermatid stage is shown in Fig. 33; here there is a large plasmosome, and closely associated with it in the center of the cell a large mass of chromatin from which strands of varying thickness radiate to the nuclear membrane. In a somewhat later stage (Fig. 34) the central clump of chromatin has all disappeared, and the nucleus contains only a faintly staining reticulum. In the half grown spermatozoa, attached to the Sertoli cells, the nucleus is finely granular (Fig. 35). This figure was drawn from a preparation stained with thionin. The ring was stained a deep blue, and the nuclear granules also blue, deeper at the proximal end. These figures are given as evidence that the spermatids and spermatozoa are not visibly dimorphic, each of the figures being typical for all of the nuclei of the stage which it represents.

Summary.

1. The probable number of chromosomes in the spermatogonia of the guinea-pig is 56, and in the spermatocytes 28.

2. Synapsis is of the parasynaptic type.

3. A typical heterochromosome is present in the growth stages of the first spermatocytes. In metakinesis this is separated into a larger and a smaller component, as in many insects.

4. The second spermatocytes are visibly dimorphic in the rest stage, one containing a larger, the other a smaller heterochromosome.

5. The spermatids and spermatozoa are not visibly dimorphic.

Discussion.

One or more unpaired heterochromosomes have been reported by Guyer ('09, '10) for the guinea-chicken, domestic chicken and man, and by Jordan ('11) for the opossum. This is the first case, so far as I am aware, in which an unequal pair of heterochromosomes has been found in a vertebrate. Although it has not been possible to follow the heterochromosomes through the second maturation mitosis, there is no evidence against the supposition that every chromosome divides equally in that mitosis, giving spermatids one half of which contain a division product of the larger heterochromosome, the other half one from the smaller heterochromosome. If it can be shown that the female guinea-pig has 56 chromosomes, two of which correspond to the larger heterochromosome of the male, then the fertilization formula will be like that for similar cases in insects.

	Eggs.		Spermatozoa.		Zygote.
Gametes	$27 + X$	+	$27 + X$	=	$54 + X\,X = ♀$
	$27 + X$	+	$27 + Y$	=	$54 + X\,Y = ♂$

This is what is to be expected, but, in view of recent developments in echinoderm spermatogenesis, it is at any time possible that we may find other conditions; for example,—

	Eggs.		Spermatozoa.		Zygotes.
Gametes	$27 + Y$	+	$27 + X$	=	$54 + X\,Y = ♂$
	$27 + Y$	+	$27 + Y$	=	$54 + Y\,Y = ♀$

In this case the female would still be homozygous but the male would have the excess of chromatin, realizing in that respect McClung's ('02) original suggestion in regard to the "accessory" in the Orthoptera; and the spermatozoön containing the X chromosome would be male-producing instead of female-producing.

It has always seemed to me somewhat improbable that the small heterochromosome of an unequal pair owes its smaller size to gradual degeneration, and much more likely that the

unequal pair may, at least in some cases, be composed of an equal pair of chromosomes with an unpaired heterochromosome fused with one member of the pair, as McClung ('05) has shown to be the case in Hesperotettix.

The definite cases of regulation in the number of heterochromosomes in the male-producing eggs of *Aphis* and *Phylloxera;* and the case of *Rhabditis nigrovenosa* (Boveri, '11; Schleip, '11) where a hermaphrodite with 12 chromosomes produces spermatozoa with 5 and 6 respectively, by rejecting one heterochromosome in the second maturation division, naturally suggest that the origin of the unpaired heterochromosome in the male may be attributed to a similar regulatory process occurring somewhere in the evolution of a hermaphrodite organism, and giving male and female-producing spermatozoa. Such a regulation might give at first males and hermaphrodites, a not uncommon condition, but degeneration of the male reproductive organs in the hermaphrodite would result in the usual bisexual condition with the sexes equal in numbers, unless the sex ratio were changed by an environment discriminating against one sex, or by some regulation in the number of male and female-producing spermatozoa. The unequally paired condition of the heterochromosomes might easily have come about by fusion of the heterochromosomes in both sexes with another pair of chromosomes in the course of a general reduction in the number of chromosomes characteristic of the species. It is much more difficult to see how the changes necessary to bring about the condition indicated in the second formula could occur, since we must account either for the disappearance of three X chromosomes leaving only the one X in the male, or for the appearance of an X chromosome *de novo*. Payne's ('09, '10) figures for several of the Reduviidae suggest other complications.

Some recent observations on other material have impressed upon me the probability that the chromatin units representing sex and other characters may be very small indeed, and that in such cases as that of *Culex* (Stevens, '11), where no heterochromosome differentiation of any kind has been detected, the members of a pair of chromosomes may differ by a sex unit or some other character unit, and the difference in size come within the probable errors of most careful observation.

Were it not for a few cases of condensation of an apparently equal pair of heterochromosomes in the male germ cells (Lepidoptera, Stevens, '06, Dederer, '07, Cook, '10; *Anisolaba maritima*, Randolph, '08), and in the female germ cells (*Aphrophora*, Stevens, '06, *Rhabditis nigrovenosa*, Boveri, '11), one might suspect that the condensed condition of the odd chromosome and the unequally paired heterochromosome of the growth stage of the first spermatocytes might be due to their unpaired or unequally paired condition preventing them from joining with the other bivalent chromosomes to form a spireme, especially in cases of parasynapsis. In *Culex* no such difference is effective in bringing about a condensed condition of one pair of chromosomes and in *Anopheles*, where the difference in size of the heterochromosomes is slight, condensation is much less complete than in most cases. In the guinea-pig condensation is complete from the synapsis stage on.

In the guinea-pig the condensation of the heterochromosome pair and its behavior in mitosis are as typical as in *Tenebrio* and many other coleoptera previously described by the author, and only the large number of chromosomes of such a consistency that good fixation in mitosis is difficult to secure, makes it impossible to give as complete a demonstration of the relation of the heterochromosomes to sex as in many other forms.

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May 23, 1911.

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